

Metagenomics for etiologic agent discovery

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Alkek Center for Metagenomics and Microbiome Research

- Facilitate/support metagenomics studies around the country.
 - Currently involved in 60+ collaborative projects
- Develop new projects aimed at deciphering host/microbe interactions and translating results to the clinic
- Provide infrastructure/expertise to explore host-microbe interactions
- Drive technology and analytical development to improve metagenomic capabilities
 - Collaborate with BCM Human Genome Sequencing Center

A Metagenomics Approach



Sample



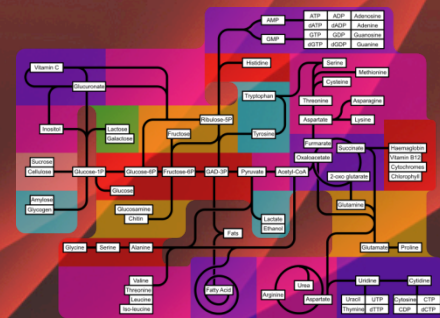
Enrich bacteria,
viruses, fungi



Extract DNA/RNA



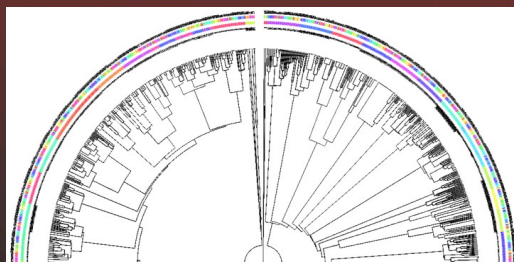
WGS
(Illumina, 454)



Genes, pathways



16S rRNA gene
fingerprint



Community Structure

cDNA library generation

Viral Metagenomics cDNA Library Generation

Total Nucleic Acid

Random RT-PCR

Primer Design
5'-(V₈AA)-3'

Tagged Random PCR

Primer Design
5'-(Seq. Adapter)-(Barcode)-(V₈AA)-3'

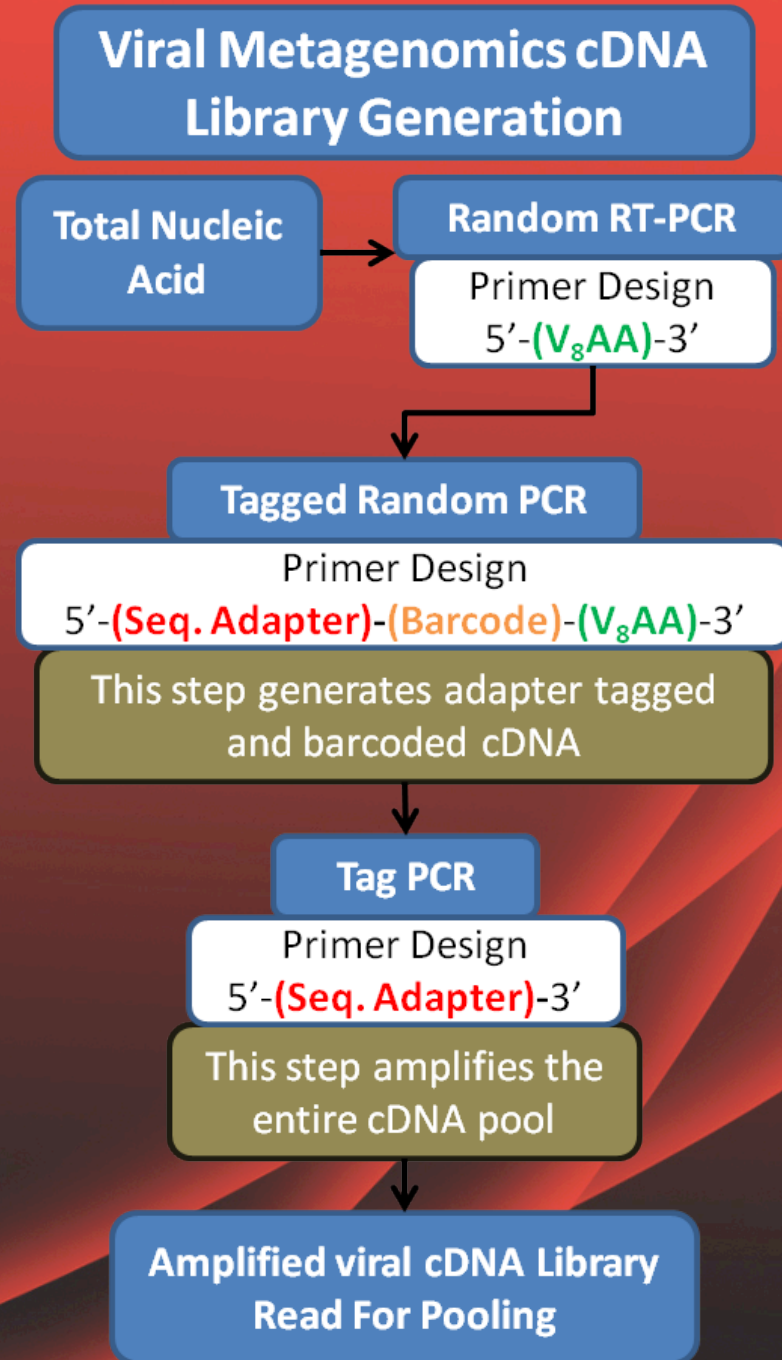
This step generates adapter tagged and barcoded cDNA

Tag PCR

Primer Design
5'-(Seq. Adapter)-3'

This step amplifies the entire cDNA pool

Amplified viral cDNA Library
Read For Pooling



The Challenge

- Early metagenomics studies:
 - Few, high-volume samples per study

- Our current studies:
 - Large number of samples



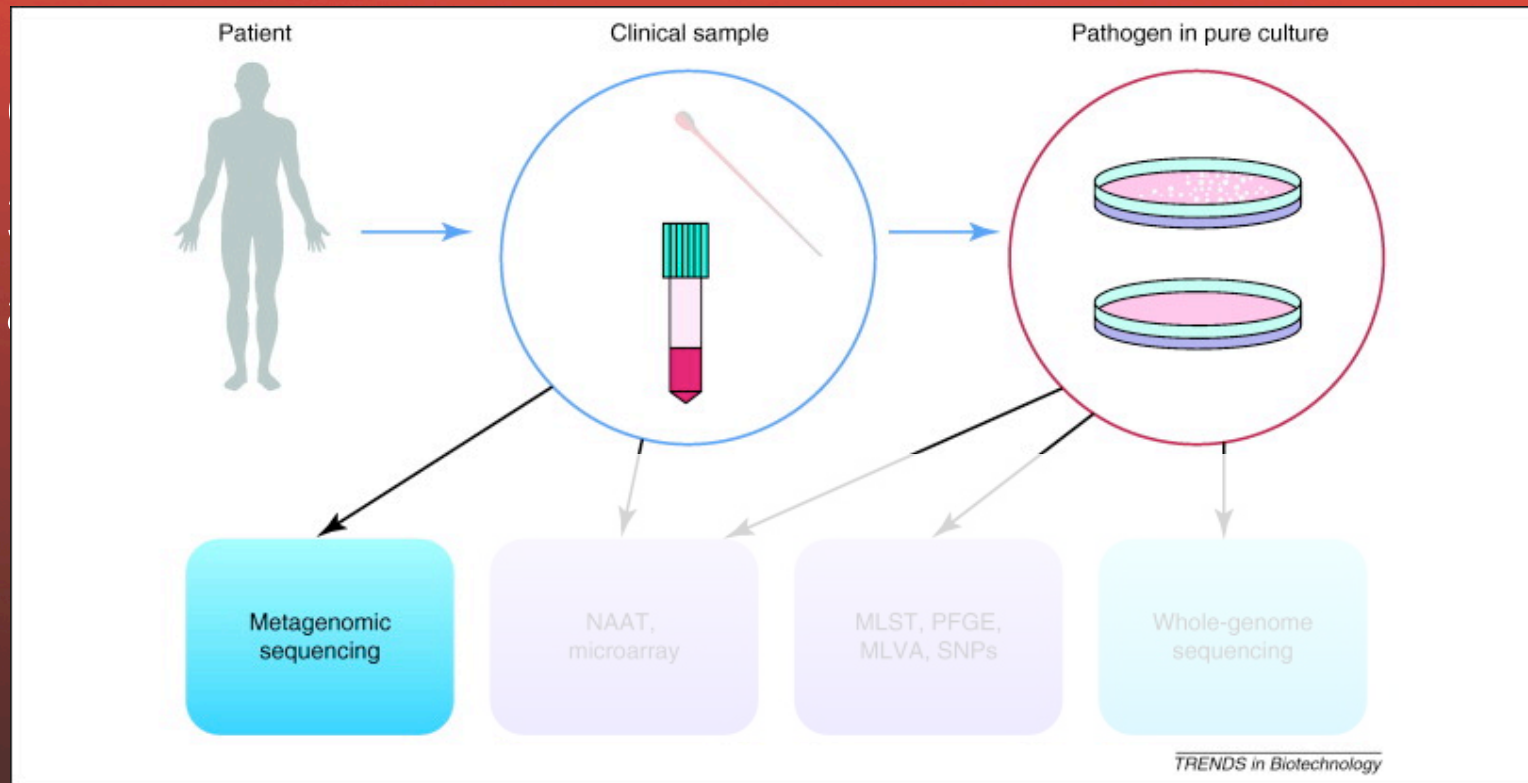
Considerations

- Sample purification techniques lead to sample loss.
- Combat high background (host) with deeper sequencing

Diseases of suspected infectious etiology

- Kawasaki disease
- Type 1 Diabetes
- Fevers of unknown origin
- Sclerosing cholangitis
- Brainerd diarrhea
- Polymyositis
- Polyarteritis nodosa
- Wegener's granulomatosis
- Behçet's syndrome
- Goodpasture's syndrome
- Takayasu's arteritis
- Eosinophilic pustular folliculitis
- Sweet's syndrome
- Type 1 Diabetes
- Kikuchi's disease
- Chronic fatigue syndrome
- Idiopathic pulmonary fibrosis
- Polymyositis
- Idiopathic pneumonia syndrome
- Still's disease
- Malakoplakia
- Behçet's syndrome
- Nephrolithiasis
- Chronic culture-negative prostatitis

Infection during chemotherapy



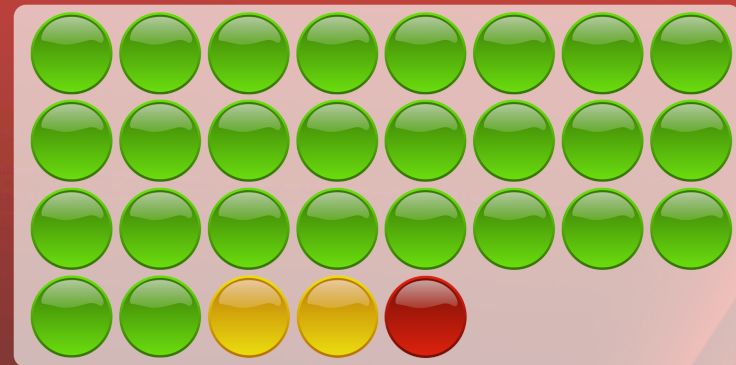
- Our approach: 16S sequencing on blood collected from chemotherapy patients with suspected bacterial infection




Efficacy

16S generated for
29 blood samples



Compared top OTU
with reported pathogen



-  Most abundant OTU matches traditionally identified pathogen.
-  16S only specific enough to match at the family level.
-  Traditional methods identified gamma hemolytic strep, 16S sequencing found staph.

Current Activity

- We are generating sequence for 40+ blood samples from patients where an infectious agent was not identified by traditional methods
- Identify organisms responsible for infection
 - Are these patients being infected by one or a few opportunistic pathogens?
 - Develop cheap diagnostic tests.

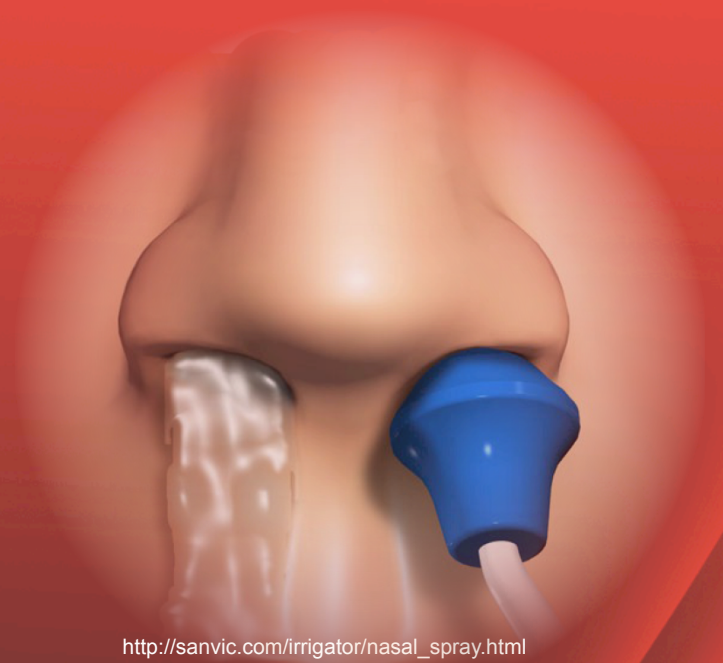
Kawasaki disease (KD)

- Affects mainly children (6mo-5yrs) of Japanese or Korean descent
- Important cause of acquired heart disease
- Typically treated with IgG and aspirin
- What causes Kawasaki Disease?
 - Infectious agent?
 - Seasonal peaks, Acute onset, Self-limited, increased susceptibility of a particular age group (toddlers), defined epidemics
 - Genetic predisposition?
 - High recurrence within families (10-15 times greater probability)
 - Incidence rates determined by race and not geographical location

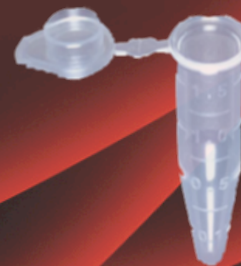


KD Samples

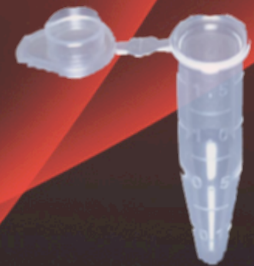
- Nasal washes:
 - 22 KD patients
 - 10 non-KD controls
- Processing:
 - Pooled into 2 groups
 - Random primed cDNA
 - Sequenced on 454



http://sanvic.com/irrigator/nasal_spray.html



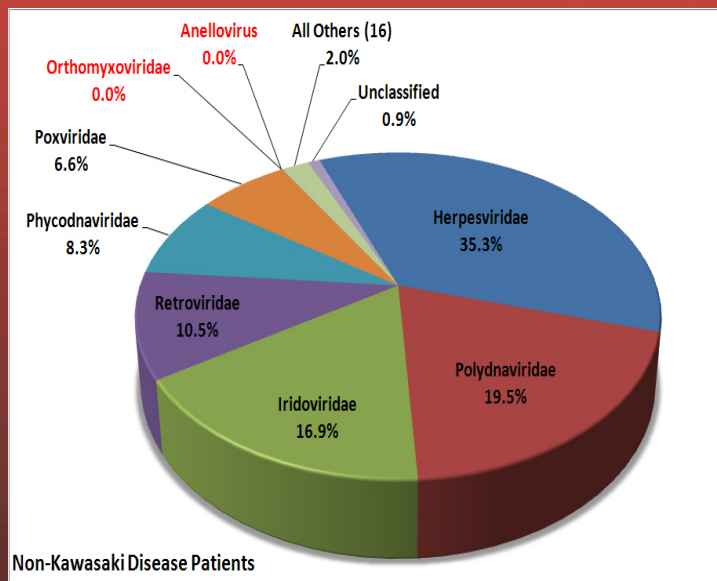
KD Patients



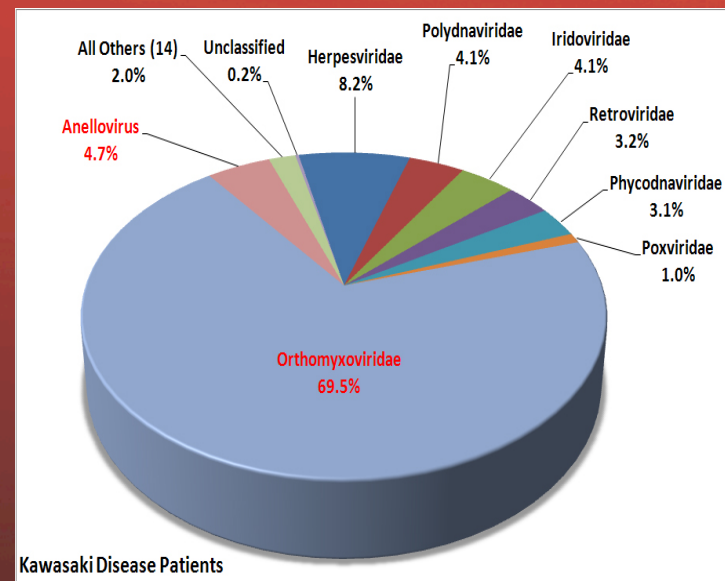
Controls

454 sequencing of cDNA

Non-KD controls



KD Patients



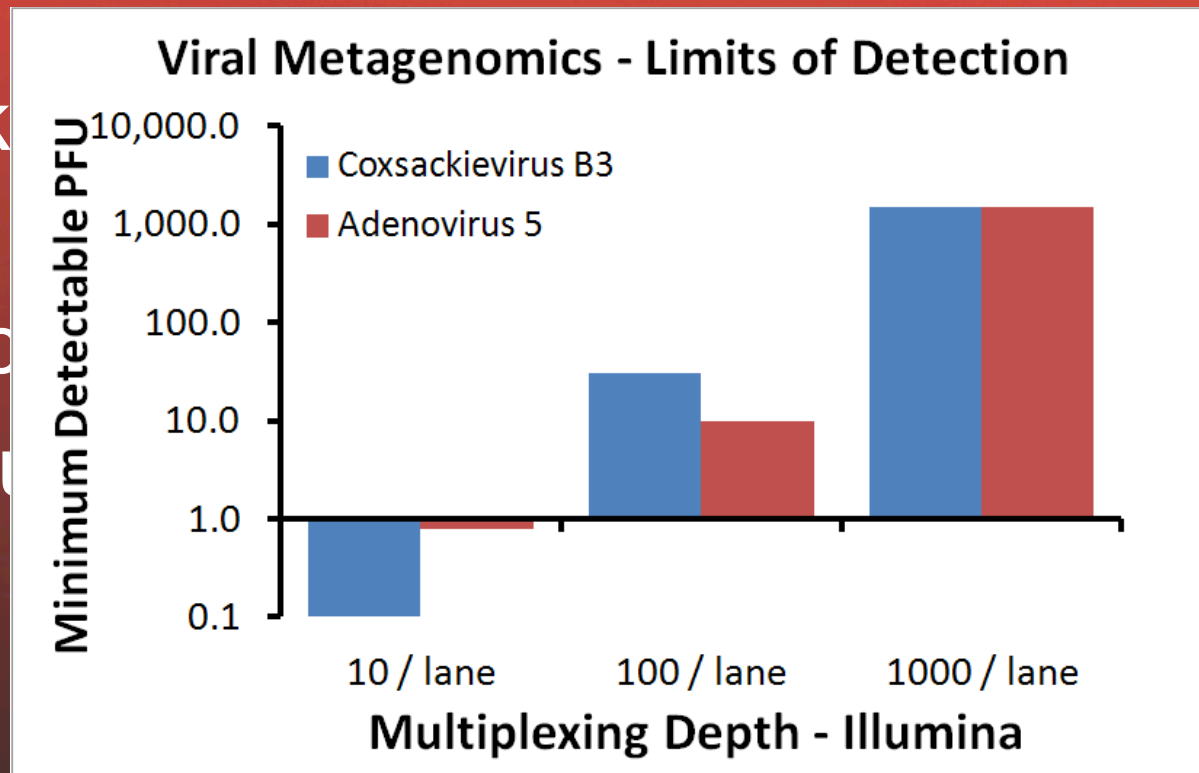
- Two viruses appear to be differentially present.
 - Direct PCR of individual samples showed one of the viruses equally present in both controls and patients. Currently testing the samples for the second virus.

Type 1 diabetes

- Results from autoimmune destruction of pancreatic beta cells
- Strong evidence for environmental trigger
 - Human enterovirus B species have been closely correlated
- We are working with nPOD and other groups to correlate these observations to metagenomic data.

Limit of Detection

- Spike
- and
- Prod
- Sequ



- Able to detect ~1 PFU or lower

Current Activity

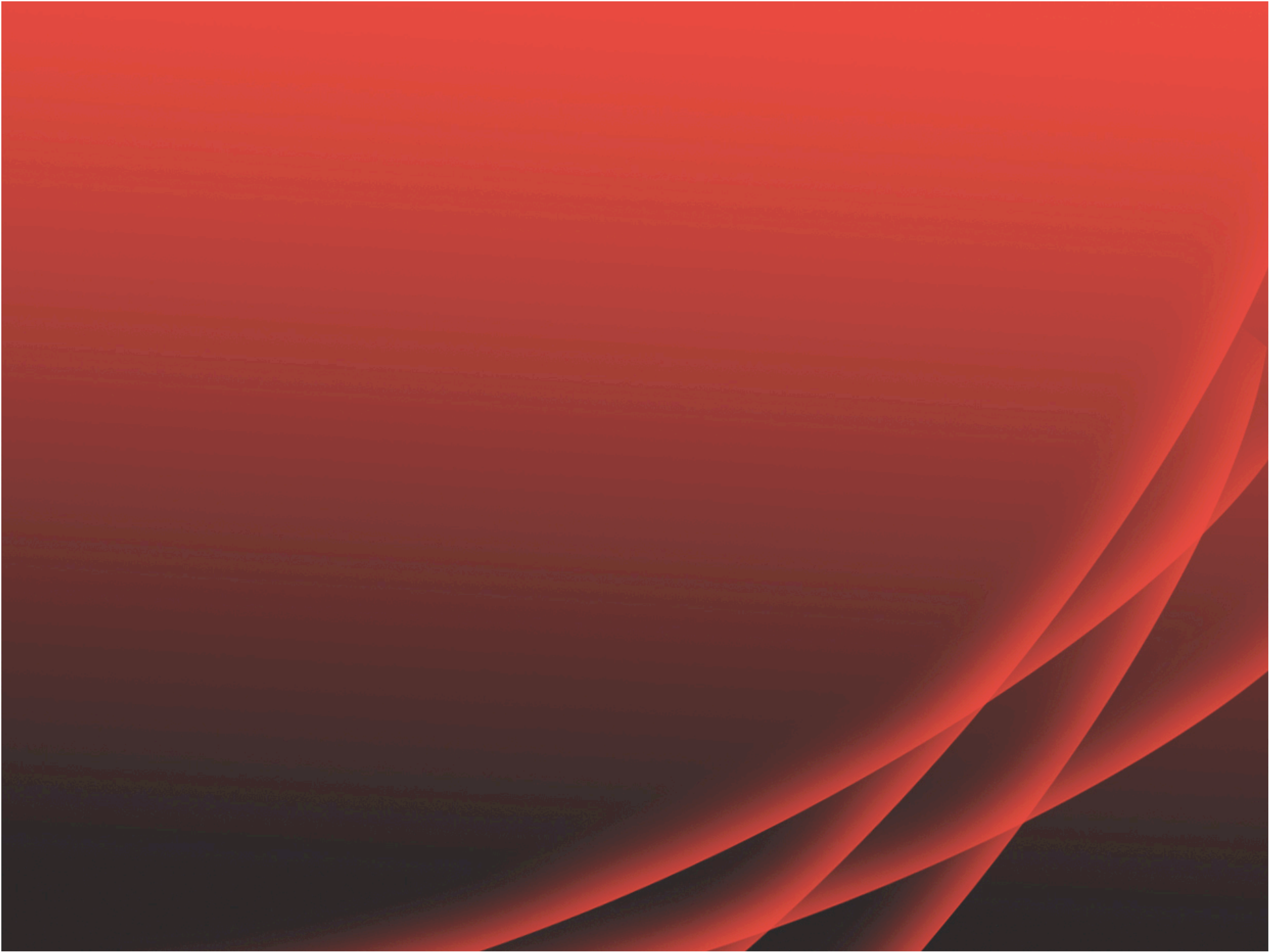
- Currently processing:
 - Pancreas
 - Spleen
 - Lymph node
 - PBMCs
- Testing various methods of depleting human RNAs.
- Screening all results for evidence of viral infection.

Why this approach is promising

- No requirement for culture or prior knowledge of the etiologic agent
- Same approach can be applied to many different types of diseases.
- Success stories:
 - Novel corona-like virus associated with diarrhea.
 - Rapid characterization of the H1N1 genome
 - Novel arena virus likely responsible for post-transplant death

Summary

- Metagenomics holds great promise for contributing to human health studies
- Fast, sensitive, sequence-independent and culture-independent
- Already lead to the discovery of dozens of novel viruses in the past decade.



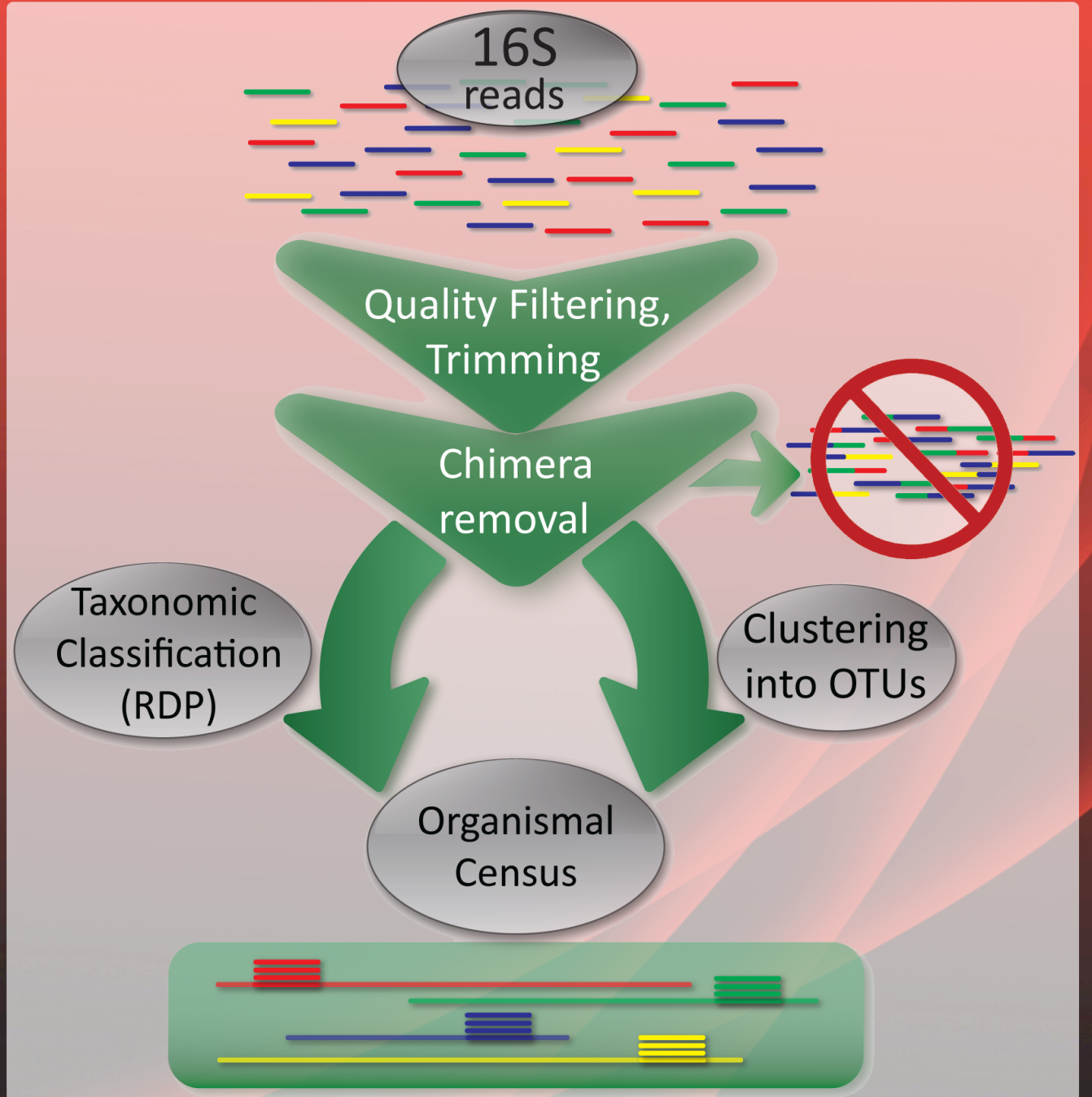
Why study the human microbiome?

- Bacterial Cells : Human Cells = 10:1
 - Bacterial genes : Human genes = 100:1
- Microbial genomes encode functions beneficial to humans
 - Production of Vitamins, e.g. Vitamin K2
 - Digestion of complex polysaccharides (plants)
- Microbiota occlude pathogens
 - Niches and food
- Impacts immune system
 - Drives neonatal immune system development
 - UPDATE*** Provides a “sparring partner” for immune system (in gut)

GLOSSARY

- **Microbiome:** The entire collection of bacteria, viruses, and fungi that naturally occupy various niches on or in a given environment.
- **Human Microbiome:** The entire collection of bacteria, viruses, and fungi that naturally occupy various niches on or in the human body.
- **Metagenomics:** Use of modern technology to study communities of microbes (microbiomes) directly in their natural environments, without the need for isolation or cultivation of the individual organism.

16S analysis



Viral Analysis

